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THE ANATOMY OF ISOETES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 126

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(WITH PLATES XIX—XXI)

There is no group of plants among pteridophytes whose anatomy has occasioned so much discussion as the genus *Isoetes*. The most recent writers, FARMER on *I. lacustris*, R. WILSON SMITH on *I. echinospora*, and SCOTT and HILL on *I. Hystrix*, disagree in many points. In view of the lack of harmony, both of observation and interpretation, it has seemed advisable to make a comparative investigation of the anatomy of several American species.

Historical

The literature on the anatomy of *Isoetes* began in 1840 with a paper by VON MOHL (24). Although he recognized the lycopod affinities of *Isoetes*, he noted several important points of difference, in the arrangement and structure of the roots, and in the nature of the cambium products. Ever since that day the question of the nature of the cambium activity has afforded a fertile field for discussion, and it is with that subject that this paper is chiefly concerned. VON MOHL regarded the whole of the secondary growth as parenchymatous, and states that, as in other vascular cryptogams, there is no increase in thickness of the wood. The next significant work was that of HOFMEISTER (16) in 1857. In discussing the cambium products he says (p. 361): "The effect of the yearly renovation of the cambial layer is not only to increase and renew the cortical tissue, but new spiral cells also become added, although only sparingly, to the wood of old vigorous plants. Individual cells of the cambium, separated by two or three cambium cells from the older principal mass of the wood, often exhibit thickenings of the walls, which by their delicacy and want of color betray their undoubted recent origin." In 1873 appeared Russow's *Vergleichende Untersuchungen der Leitbündel-Kryptogamen* (18), with a discussion of the anatomy of *I. lacustris*, *I. echinospora*, and *I. Hystrix*. This paper has had great influence on subsequent work, and to Russow's work may be attributed the cur-

rent conceptions of the nature of the cambium products, i. e., that the cambium produces cortex externally to itself, but internally it produces a tissue which he calls the "prismatic layer," and this he regards as being made up of phloem cells, tracheids, and parenchyma. HEGELMAIER (12), writing in 1874, is inclined to question Russow's interpretation, but adds that no decisive grounds can be given against the interpretation of the *Dauerzellen* as soft bast. FARMER (9) published in 1890 a very full discussion of *I. lacustris*. He seems reluctant to accept Russow's theory of the nature of the cambium products, but does not offer another. After quoting Russow he adds: "Quite apart from the fact that it is produced internally to the cambium and would, from the point of view of its position, be anomalous, its structure is remarkably complex and heterogeneous." In discussing the structure of the "prismatic layer" he says:

The zone-like arrangement consists in alternations of tubular thin-walled cell-rows of varying thickness, whose cell contents are clear and watery, with others, whose cells are wider in the radial direction, and filled densely with starch. Occupying a middle position in the latter zone is embedded an irregular ring of cells whose walls are thickened like those of the tracheids, but these too, unlike the latter, often contain protoplasm and starch.

In 1900 R. WILSON SMITH (22) discussed the morphology of *I. echinospora*, with some incidental work on its anatomy. His observations do not agree with those of FARMER on *I. lacustris*, and he proposes "to drop the term phloem until its justification is established on physiological grounds." In the same year there appeared a very exhaustive paper on *I. Hystrix* by SCOTT and HILL (19). The nature of the cambium products was taken up, and Russow's conception was indorsed, in that they regard the secondary tissues internal to the cambium as consisting of secondary tracheids, parenchyma, and phloem. Their statement is as follows:

The cambium in *I. Hystrix*, arising in the tissues just outside of the xylem cylinder, continues its activity indefinitely, producing parenchyma, phloem, and a variable amount of secondary xylem on its inner side, and secondary cortical parenchyma only, towards the exterior.

Each of the last three writers has based his conclusion on the study of a single species, while Russow, who investigated three forms, was hampered by the fact that he worked on herbarium material. The

desirability of a comparative study of several species embracing a considerable range of habit is obvious.

Material

I am indebted for material of *I. Tuckermanni* var. *Harveyi* (A. A. Eaton) Clute (*I. Harveyi* A. A. Eaton) to Dr. LEROY H. HARVEY, who made a collection at Pushaw Pond, Maine, of plants in various stages of development; to Mr. WILHELM SUKSDORF for material of *I. Nuttallii* Engelm. (*I. Suksdorfii* Baker) collected at Bingen, Wash.; and to Dr. H. C. COWLES for material of *I. echinospora* var. *Flettii* which he collected at Spanaway Lake, Wash., in the summer of 1907. The latter material has been kept in cultivation at the greenhouses of the University of Chicago and has afforded abundant material for the study of a close series of stages in the development of the stem. I am also indebted to the late Mr. A. A. EATON of the Ames Botanical Laboratory, North Easton, Mass., for the determination of *I. melanopoda* Gay.

For killing and fixing the stems a medium solution of chrom-acetic acid was used. The material was embedded in paraffin and cut in serial sections 7 to 12 μ thick. Some of the series were transverse and others longitudinal, both parallel and perpendicular to the furrow. A considerable number of stains and combinations of stains were used, of which the most satisfactory were the following: a combination of safranin and anilin blue devised by Dr. LAND; iodine green and Bismarck brown; iodine green and eosin.

The four species studied present a considerable range in habit. *I. Tuckermanni* var. *Harveyi* is a submersed form which grows in water 30–150^{cm} deep. The trunk is deeply two-lobed, occasionally three-lobed. The leaves are short, strongly recurved, without stomata, and said by Eaton (8) to be relatively the stoutest of any North American species, being 5–6^{cm} in length and 2.5–3^{mm} in diameter. *Isoetes echinospora* var. *Flettii* grows on the gravelly edges of mountain lakes in Washington. In the spring it may be submersed, but during most of the year it is out of the water. The gravel holds water so that the substratum is never very dry. The trunk is two-lobed and the leaves are moderately stout. The largest form studied is *I. melanopoda*. It has a large two-lobed trunk, with

very long, moderately stout leaves. It grows in inundated fields and shallow ponds, where it is emersed during most of the summer. *I. Nuttallii* has a three-lobed trunk and long, very slender leaves. It never grows under water, but near springs or springy places, and on low wet grounds or meadows, where the ground becomes very dry in the latter part of the season.

Investigation

The complicated structure and arrangement of the old stem is more easily understood by a study of the sporelings. The stem of a sporeling is a mass of undifferentiated parenchyma, traversed by leaf traces, which come together to form the short flat stem plate and continue outward and downward into the roots. All observers agree that in the young plants there is no cauline portion in the stem bundle, but whether or not there is a cauline portion in the older stems has long been a disputed point. HOFMEISTER, DE BARY, CAMPBELL, and FARMER look upon the stem bundle as being made up of leaf traces; while HEGELMAIER, BRUCHMANN, and SCOTT and HILL maintain that there is a cauline portion. In the short and compact stem there are no indications of a procambial strand, and as there are never any tracheids present above the last leaf trace, the evidences of a cauline portion are not satisfactory. In following the cross-sections of leaf traces in a series of longitudinal sections up to the place where the traces coalesce, it is easily seen that there is a sufficient amount of tracheary tissue in the leaf traces to account for all the xylem in the stem bundle. SCOTT raised the objection that the tracheids of the stem are unlike those of the leaves, but there is no greater difference between the tracheids of the stem and of the base of the leaf, than between those of the base of the leaf and of the upper part. The difference is chiefly one of length, and this, together with differences in the distribution of the thickening of the walls is correlated with the rate of growth of the part in which the tracheids are found.

In all plants there is a separation into root and stem regions, and this becomes more marked as the plant enlarges. This suggests that the tuberous body is not wholly stem, but a contracted stem and main root. Owing to the compact growth and the consequent displacement of tissues, some of the root bundles in an old plant may be found higher

up in the cortex than the old leaf traces. In fact it is not uncommon, in sections of old stems cut at the very apex of the stem, to find in the cortex a longitudinal section of a root making its way out. Hence, although the stem and root regions are sharply marked off in the central axis, it is impossible to delimit these regions in the cortex. In the young plant shown in *fig. 4*, all the leaf traces and root bundles are functional. In the plants shown in *figs. 19, 20*, all the roots are dead except a few at the bottom, and all the leaf traces except a very few at the top. As the plant increases in size, the old leaf traces are unable to keep up with the growth of the stem and are torn apart, leaving the old stumps attached to the central axis, while the rest of the leaf trace is carried out farther and farther by the growth of the cortex, and in time is sloughed off.

The arrangement of the roots is described in great detail by SCOTT and HILL for *I. Hystrix*, a three-lobed form. The two-lobed trunks show no essential variation from that type, and it is therefore not necessary to give a detailed discussion of the subject. *Fig. 6* illustrates the appearance of the lower part of the stem in cross-section, showing the relative position of the roots of various ages. The roots near the center, which are outlined by dots, are those in meristematic condition; next to them are the mature active roots which are indicated by shaded circles; beyond the mature roots are those which are dead and crushed. The relation of the sets of roots is indicated in *fig. 19* also. The arrangement of the roots in the two-lobed species was first correctly worked out by HOFMEISTER; the youngest sets of roots are those nearest the furrow; and of those in the furrow, the youngest are at the ends.

THE ROOT

The structure of the roots has been described so many times that it is unnecessary to take it up in detail. The most interesting feature is that they are collateral and monarch, without showing any traces of reduction. As the root bundle passes out from the vascular axis it is a round or flattened bundle surrounded by parenchyma. At a short distance from the stem, phloem replaces the parenchyma on the side away from the center of the stem. The phloem is always limited to one side and is small in amount in comparison with the xylem. There is also proportionally much less than in the leaf traces. The

protophloem and protoxylem are usually differentiated about the same time, but the protophloem occasionally precedes the protoxylem. The complete differentiation of the phloem is usually complete before that of the xylem. The differentiation of the xylem in the roots is much slower than in the leaf traces. *Fig. 7* illustrates the structure of a root bundle before it is completely developed and while it is still in the cortex. The eccentric position of the bundle in mature roots is shown in *fig. 8*. While the root bundle is making its way through the cortex the endodermis is poorly differentiated, but in the mature root outside of the stem it is well defined. The pericycle is poorly developed. It is usually entirely absent opposite the phloem, but opposite the xylem there is usually a small amount.

THE LEAF

The leaves of the four species studied present considerable variation, both in general appearance and anatomy.

The leaf of *I. echinospora* var. *Flettii* is moderately stout, and quadrangular in outline (*fig. 9*). In the cortex and lower part of the leaf the bundle is fairly well developed, but above the sporangium it becomes a slender strand. The bundle is collateral throughout, but tends to become concentric in the upper part. The differentiation of the phloem regularly precedes that of the xylem, as was found by KRUCH (17) in the species which he studied. The protoxylem in most cases is plainly exarch, but sometimes the differentiation is nearly simultaneous. In this species I found no cases of distinctly mesarch protoxylem. It is common for the metaxylem to develop in three directions from the protoxylem, but I found no cases in which it developed on the fourth side, i. e., on the side toward the phloem, although KRUCH and SCOTT and HILL find that in their species the bundle tends to become mesarch in the region of the sporangium. There is evidently a certain difference in this respect in different species, for in *I. Nuttallii*, which I shall discuss later, mesarch protoxylem is sometimes present. While the protoxylem and metaxylem are both composed of spiral and annular tracheids, or tracheids with irregular spirals, the protoxylem can usually be recognized by the fact that the spirals and rings are looser. The protoxylem cells of the lower part of the leaf are small and are the

first to show signs of crushing, but those in the upper part are large and later form canals by the loss of the thickened portions. In the upper part of the leaf the protoxylem is usually limited to a single large vessel, whose place can be recognized in old leaves by the large canal formed by its disappearance (*fig. 13*); but in the lower part of the leaf, as well as in the leaf trace, the protoxylem cells are smaller, several in number, and less sharply marked off from the metaxylem. *Fig. 10* represents the section of a young leaf near the tip, in which the only indication of the protoxylem is the large central cell, which is not yet thickened, while the phloem is represented by several cells. The phloem consists of very long and slender tubes whose sieve plates are terminal. They come out clearly in sections stained in anilin blue or Bismarck brown. Although several preparations suggest the presence of thin areas on the lateral walls, the evidence is not conclusive. In the portion of the leaf above the ligule there is a very abrupt decrease in the amount of xylem, but not a corresponding decrease in the phloem. The phloem is well developed throughout, but instead of forming a band on one side of the bundle, as in the lower part of the leaf, it forms an arc. The phloem does not disappear from the center of the arc, leaving two lateral groups such as KRUCH has described in *I. Hystrix*, *I. Duriaei*, and *I. velata*, but the amount in the center of the arc is variable. Near the top of the leaf the phloem is sometimes most abundant in the middle of the arc. In *fig. 14* is shown a leaf trace from the cortex at the base of the leaf. This is the type of bundle which is found in the leaf trace as it traverses the outer part of the cortex, and in the leaf itself from its base to the ligule. Just above the ligule, the bundle passes abruptly into the reduced type of *fig. 13*. In the former, the tracheids, which are fairly uniform in size, are scattered among parenchyma cells that have abundant protoplasm and large nuclei. Before the sporangium is mature, the outer vessels, the protoxylem, begin to collapse. In this type of bundle the phloem is always in a band and shows no tendency to surround the xylem. The amount of phloem is less than in the upper part of the leaf, and by the time the leaf is mature it is almost entirely obliterated by crushing. In following the bundle through the cortex toward the center of the stem the first change seen is a condensation of the bundle by a reduction in the amount of paren-

chyma, although a few parenchyma cells usually remain in the center as long as the leaf trace can be identified (fig. 15). For some distance the compact bundle remains distinctly collateral, but the phloem decreases in amount, and near the stele disappears entirely, the leaf trace consisting of a strand of xylem with a few parenchyma cells in the center and a parenchyma sheath. The xylem cells become shorter, and it is no longer possible to distinguish between protoxylem and metaxylem. The next stage is the merging of the tracheids and parenchyma of the various leaf traces to form the central axis.

While in general the leaf traces of the other species are similar to those of *I. echinospora* var. *Flettii*, they present certain interesting variations. The leaf of *I. Tuckermanni* var. *Harveyi* is also quadrangular, but is shorter and more rigid. The bundle, however, is not so well developed, either in amount or structure. The tissues are not well differentiated; the phloem in particular is much less sharply differentiated than in the other three species. The thickening of the walls is slight and sieve plates are not evident. The amount of xylem is noticeably less, but the phloem, although not abundant, is not so much reduced as the xylem. In the lower part of the leaf the phloem is a narrow band, but in the upper part of the leaf it forms an arc and tends to surround the xylem. The formation of sieve cells begins in the middle of the band, or arc, and extends around three sides of the xylem. The phloem in the center of the arc is small in amount and more rudimentary than that on either side, so that in places there are indications of a tendency to form two lateral groups. The sieve cells in the lateral groups are smaller, as well as more numerous, and they continue to function long after the first-formed cells have become crushed and functionless. In this species the sporangia are comparatively small.

I. melanopoda has very long, moderately stout leaves, with large sporangia. The leaves have a greater diameter than those of *I. Tuckermanni* var. *Harveyi*, but they are very much longer and relatively less stout. The amount of xylem is greater, and the bundle is in general stronger than in the two preceding species. The leaf trace in the cortex of the stem contains on an average twice as many tracheids as that of *I. Tuckermanni* var. *Harveyi*, while in the leaf trace above the sporangium there may be three to eight tracheids

instead of one to four, as in *I. echinospora* var. *Flettii* or *I. Tuckermanni* var. *Harveyi*. As in the other species, the phloem forms an arc in the upper part of the leaf. As the phloem is abundantly developed at the sides of the arc and poorly developed in the middle, there is a tendency for it to separate into two lateral groups.

The leaves of *I. Nuttallii* differ considerably from those of the other species, both in external appearance and structure. They are triangular in outline, long and very slender, indeed almost thread-like. The sporangia are extremely large, while the leaf tissue, in the sporangium region as well as above, is notably small in amount. The bundle, however, is well developed and larger than in any of the preceding species. It differs conspicuously from those of the other species in the large amount of xylem present in the bundle above the sporangium and in the upper part of the leaf. Although, as in the other species, the bundle becomes reduced above the sporangium, there are usually eight or ten tracheids and sometimes fifteen or sixteen present for a considerable distance above the sporangium. In the region above the sporangium, as in the other species, the protoxylem consists of large cells, which are later replaced by canals. The walls lining the canals are heavily lignified. There is no trace of an endodermis, such as SCOTT and HILL find in *I. Hystrix*. The protoxylem in this region is occasionally mesarch, a few tracheids of the metaxylem developing on the side toward the phloem, although the greater amount of metaxylem is always on the adaxial side of the leaf, where it frequently forms a narrow band. In the region of the sporangium the metaxylem is in the form of a crescent, with the heaviest development often at the ends of the horns. In such cases the bundle tends to become mesarch. The phloem also is well developed and abundant, the sieve tubes regularly separating above the ligule into stout strands. A transverse sieve plate is shown in *fig. 11*.

If the four species are arranged in a series according to the size and development of the leaf traces, the series is as follows: *I. Nuttallii*, *I. melanopoda*, *I. echinospora* var. *Flettii*, *I. Tuckermanni* var. *Harveyi*. If the species are arranged according to habitat, from terrestrial to aquatic, the order would be the same, with *I. Nuttallii* as the most terrestrial form and *I. Tuckermanni* var. *Harveyi* as the most aquatic. A series arranged according to the size of the leaves is as follows: *I.*

melanopoda, *I. Tuckermanni* var. *Harveyi*, *I. echinospora* var. *Flettii*, *I. Nuttallii*. A series according to the size of the sporangia is as follows: *I. Nuttallii*, *I. melanopoda*, *I. echinospora* var. *Flettii*, *I. Tuckermanni* var. *Harveyi*. It is perhaps not safe to generalize from a comparison of only four species, but it is worthy of note that the size and development of the bundle in these forms is not related to the size of leaf, but follows the other two series, that of habitat and size of sporangium.

HILL (13) calls attention to the presence of two canals in the leaves of *I. Hystrix*, which he regards as representing the parichnos of the *Lepidodendreae*. In none of the four species examined is there any trace of a canal or any indication of a tendency to form canals. In this respect these species agree with *I. lacustris*.

THE STEM

As is well known, the stem of *Isoetes* is a short tuberous body, whose vascular axis is very small in proportion to the diameter of the stem. The stem grows in length very slowly, and the apical region is left in a deep pit by the overgrowth of the surrounding region. If the term stele may be applied to a region whose connection with a plerome is far from certain, then the vascular axis may be defined as a non-medullated monostele, consisting of xylem and parenchyma forming a loose network. The xylem is made up of short spiral, annular, and netted tracheids, whose long axis is transverse to the stem (*fig. 22*). The parenchyma cells contain abundant protoplasm, and the nuclei appear active. The xylem axis is surrounded by similar parenchyma cells, one to three layers deep, but in neither young nor old plants is there a trace of phloem. A great many young plants of *I. echinospora* and a few of *I. Tuckermanni* var. *Harveyi* were examined, and in all cases the xylem is surrounded by undifferentiated parenchyma. In very young plants the vascular axis is exceedingly small, but there is a constant increase both in length and diameter with the increase in the number of leaves.

The differentiation into protoxylem and metaxylem, which is usually well marked in pteridophytes, is lacking in *Isoetes*. There is neither a difference in the time of development nor in the character of the elements. A difference in character of elements is not likely to

occur in such a slow-growing stem, and a difference in time would be difficult to detect in a stele whose elements run transversely.

SCOTT and HILL have claimed a slight differentiation in time in certain cases, giving as evidence sections which show tracheids at the outside and parenchyma in the center. But since the xylem is made up of leaf traces which curve down from the leaves, as in *fig. 4*, it is evident that it would be possible to obtain a section which shows this condition without being an example of protoxylem differentiation. In young stems it is not uncommon to find that at least one section shows this apparent differentiation into protoxylem and metaxylem. As was mentioned above, an examination of a series of cross-sections of leaf traces as they approach the central axis indicates that there is no differentiation into protoxylem and metaxylem in that part of the leaf trace. Even if it were present in the leaf traces, the transverse arrangement of the tracheids would cause it to appear in tiers rather than in vertical strands, as in other pteridophyte stems.

The cambium, which appears very early (*fig. 3*), begins its activity in the parenchyma which surrounds the central axis, so that all the tissues which are found outside this thin layer of parenchyma are secondary. The secondary tissues of *Isoetes* have always been described as anomalous, and so have furnished a fertile field for observation and theorization. The cells which the cambium cuts off externally are ordinary thin-walled parenchyma cells, which have always been called cortex. Whether they represent ancestral phloem is, of course, an interesting question, but there is nothing in their structure to suggest an answer. This is the great storage region of the plant, and the amount of this tissue is much greater than that formed internally by the cambium. In the middle and outer regions of the cortex the cells become rounded, often lobed, as in the mesophyll of leaves (*fig. 18*), and always contain large amounts of starch. The cortex increases its thickness from year to year, although there is a continual loss by the sloughing-off of the outer layers. In the outer region there is little or no starch.

The tissue formed internally to the cambium, the so-called "prismatic layer," is that which has aroused the greatest interest. In a freshly cut section this layer is a glistening white and stands out sharply from the surrounding region. In stained sections it is seen

that the "prismatic layer" is composed of several kinds of cells. In the four species studied there is a considerable range both in the types of cells and in their distribution.

In *I. echinospora* var. *Flettii* there is found scattered irregularly a rather large number of active nucleate parenchyma cells, whose walls are slightly thickened. Associated with them are other cells which are almost or entirely empty and whose walls are not uniformly thickened, but have round, oval, or irregular pits. In such cells the thickening is not very heavy. There are usually other cells in which the thickening is more pronounced and is arranged in irregular bands or rings. In some cells there are heavy bands in addition to the irregular pittings.

Of the four species, *I. Tuckermanni* var. *Harveyi* most closely resembles the preceding species, but it differs in several points. There is little or no active parenchyma, the entire tissue consisting of cells whose walls are thickened irregularly. These cells are seldom entirely empty, but usually contain a little protoplasm and small, apparently degenerating, nuclei. The cells are for the most part of the type shown in *fig. 23*, in which the pits are small and irregularly distributed, and there are but faint indications of banding. In the older plants, however, it is not uncommon to find the thickening forming more or less definite bands. In the older parts of old plants the pits become smaller and less prominent, tending to disappear entirely. Sometimes the pits can be seen in sections stained in Bismarck brown, when with a less transparent stain, such as Delafield's hematoxylin, the pits cannot be distinguished, and the walls appear to have a uniform and rather heavy thickening. Apparently the thickening of the cell walls goes on through a period of several years, the first thickening being irregularly distributed, leaving irregular pits, while the later deposits include the whole of the wall and tend to obliterate the pits.

In *I. melanopoda* we have all the types of cells which have been described for *I. echinospora* var. *Flettii*: active parenchyma cells, pitted cells, and cells with irregular thickened bands or rings. The pitted and banded cells are as a rule entirely empty. In addition to these types of cells, however, there are other cells with banded thickenings which are slightly lignified, and also tracheids with spiral or annular thickenings, whose lignification is pronounced, although

not as heavy as in the tracheids of the central axis or of the leaf traces (*fig. 21*). The parenchyma cells, however, have thinner walls and are richer in protoplasm than in the two species just described. The parenchyma and the various types of thickened cells are in most cases distributed irregularly, as is shown in *fig. 26*, although occasionally in the older parts of old stems there are indications of zonation.

I. Nuttallii shows the same type of cells as *I. melanopoda*, but there is a difference in arrangement. This species always shows the zonation which has been described by FARMER for *I. lacustris* and by HEGELMAIER for *I. velata* and *I. Duriaei*. The parenchyma cells form layers one or more cells thick, which alternate with layers composed of the various types of thickened cells (*fig. 25*). The zonation is evident in young plants and is very striking in old plants, especially in the older regions of the "prismatic layer." The thickened cells are usually entirely empty. The parenchyma cells of *I. Nuttallii* are larger, contain more protoplasm, and have thinner walls than those of the first two species. The parenchyma is more like that of *I. melanopoda*, although on the whole the cells are richer in protoplasm than those of the latter species. In the older parts of the stem the thickened empty cells are usually collapsed, so that the zones of thick-walled cells, which alternate with the well-developed parenchyma zones, are apparently much narrower.

Almost all the writers on Isoetes have called attention to the presence of the fine-grained starch in the cells of the "prismatic layer." This was first noted by HEGELMAIER in *I. velata* and *I. Duriaei*, in which the starch-containing parenchyma cells form zones alternating with zones of empty cells. FARMER records the presence of starch in the prismatic layer of *I. lacustris*, in which the starch-filled cells are also arranged in zones. The disposition of the starch was found to vary with the species. In *I. echinospora* var. *Flettii* and *I. melanopoda*, it is present abundantly in the cortex but not at all in the "prismatic layer." In *I. Tuckermanni* var. *Harveyi*, in addition to the starch in the cortex, there is starch in the parenchyma in the vascular axis, and in the layer of parenchyma surrounding the axis, but there is none in the "prismatic layer." *I. Nuttallii* shows what seems to be the more common arrangement in the forms previously described; that is, there is abundant starch in the "prismatic layer."

It should be noted that the only one of the four species which contains starch in the "prismatic layer" is the only one which shows well-marked zonation, and is also the one in which the parenchyma cells are the largest and contain the most protoplasm.

Discussion of secondary thickening

A study of the structure of the cells composing the "prismatic layer" is of interest only as affording a basis for an interpretation of the nature of the layer. The interpretation which is accepted in the most comprehensive of all recent works on pteridophytes, BOWER'S *Origin of a land flora*, as well as in CAMPBELL'S *Mosses and ferns* and other current texts, is RUSSOW'S theory, which more recently has received the indorsement of SCOTT. As was mentioned above, they look upon the "prismatic layer" as a complex of tissues, consisting of parenchyma, phloem, and xylem. Among recent writers SMITH is the only one who has even suggested that the tissue may be of a less extraordinary nature.

The position of this layer would naturally lead to the conclusion that it is secondary xylem, but for the fact that its composition is not what we have been accustomed to look upon as characteristic of that tissue. The parenchyma is more abundant, while well-defined tracheids are not only few in number in most species but exceedingly rare or entirely absent in others. In addition to the parenchyma and tracheids, there are the pitted cells, which have been regarded as phloem. It may be well at this point to consider the evidence upon which this claim is made. SCOTT and HILL say:

The phloem elements have an extremely characteristic structure of their cell walls which comes out conspicuously in sections stained in hematoxylin. Their walls are much pitted, the thicker bands of membranes between the pits forming a lattice-like reticulum. The pits are often subdivided by fine bars into smaller areas. Little of the nature of formed contents can usually be detected, but sometimes small, deeply staining globules are found adhering to the walls, and apparently localized at the pits. In the older parts of the stem the phloem is to a great extent obliterated, dense masses of callus-like substances appearing on the cell walls and almost filling the cavity. The masses stain like callus with coralline-soda, but the other callus reactions tried did not give wholly satisfactory results. We have not investigated the more minute histology of the phloem and thus have not demonstrated the perforations of the thin-walled areas. That may be left to other investigators, but in the meantime, we can scarcely doubt that these enucleate

elements, with the characteristic areolations of their walls, and their agreement in various reactions with the sieve tubes of the leaf, with which we shall see they are continuous, are best to be regarded as themselves representing the sieve tubes of the stem.

If no other explanation of these structures were possible, the reasons given above might be accepted as sufficient proof of the phloem nature of the pitted cells, although the fact of a cambium cutting off both phloem and xylem from the same face is so extraordinary that one does not expect the advocates of such a theory to content themselves with leaving the burden of proof to other investigators. The only analogy is in the case of *Dracaena* and its allies, whose anomalous secondary thickening has been frequently referred to in the literature on Isoetes. Before Russow's theory made its appearance, the tissue on the inside of the cambium had been regarded as secondary xylem. This was the very natural interpretation given by HOFMEISTER in his *Higher Cryptogamia* and was accepted, apparently without question, until Russow's more critical work appeared. If one is not willing to accept the Russow theory, the natural alternative is to regard the "prismatic layer" as secondary xylem. This, of course, requires an explanation of its unusual structure. The presence of a large amount of parenchyma in the secondary wood is unusual but not without parallel, as this is the case in certain *Lepidodendreae*, e. g., *Lepidophloios fuliginosus*. The presence of unlignified pitted cells, of course, is the situation which has led to controversy, and is the chief point to be explained. However, the presence of pitted cells of a phloem-like aspect does not necessitate the assumption that the cells are phloem, since that structure is found in cells of other tissues, for example, the cortical parenchyma of *Helminthostachys zeylanica*. FARMER and FREEMAN (10) in their description of the pits in the cells of the cortical parenchyma say:

The pits are remarkable, forming, as they do, not merely simple depressions in the walls, but rather being massed together in areas like the pores of a sieve plate. Indeed they may fairly be termed pitted areolae and they do not differ essentially from the actual sieve tubes themselves in the plant.

The presence of cells of this type in tissues which do not even belong to part of the bundle indicates that pitted walls are not necessarily to be taken as an indication of phloem. The usual phloem

tests applied to the "prismatic layer" do not give any positive results. In the case of the cells in question it can be shown that not only are they not phloem but that they are xylem.

A careful examination of the "prismatic layer" of such forms as *I. Nuttallii* and *I. melanopoda* will reveal the fact that, while the secondary tracheids are far removed in appearance and staining reactions from the pitted cells, an almost perfect transition series exists between the two types. Several stages are shown in *fig. 21*. It is possible to trace a series from the tracheids with lignified spiral or annular thickenings, through those with less regular thickenings and with a smaller amount of lignin, to those in which the thickening is very irregular and which have no trace of lignin. A combination of safranin and anilin blue was found to be particularly valuable in revealing slight amounts of lignin. With this variation in wall thickening and lignification, there is correlated a variation in the amount of cell contents. The existence of the transitional stages leads almost inevitably to the conclusion that the various types of cells of the "prismatic layer" differ essentially only in their stage of development, and that the layer accordingly consists of mature tracheids, immature tracheids, and parenchyma. While the series in *I. echinospora* var. *Flettii* is less perfect, it is very suggestive, but that of *I. Tuckermanni* var. *Harveyi* is usually too limited to afford much of an indication of the nature of the pitted cells.

The recognized steps in tracheid development are as follows: the more or less regular thickening of the wall; the loss of cell contents; and the lignification of the wall. It should not be assumed, because a parenchyma cell undergoes the first steps of the changes which would lead to the formation of a tracheid, that there is any inherent necessity for their continuance. It is perfectly possible that the course of development might be arrested at any point, and that any or all of these changes might be incomplete, according to the usual standards of completeness. Unfortunately, the cases of secondary growth in modern pteridophytes are so few in number and so limited in extent that there are very few opportunities for comparison; but this very thing—the incomplete development of the tracheids—has been described as characteristic of the secondary xylem of other pteridophytes, and indeed is almost made the test of secondary xylem.

BOODLE (1), in his account of the secondary thickening in the roots of *Ophioglossum vulgatum*, figures both a cross and a longitudinal section of secondary tracheids, which he describes respectively as a "developing tracheid with its protoplasmic contents" and as a "longitudinal section showing part of the xylem with one developing tracheid containing protoplasm and a nucleus." In both these cases the tracheid character of the cell is too pronounced to be questioned. In the case of *Angiopteris evecta*, HILL (14) says with reference to the secondary growth: "Semi-lignified elements with protoplasmic contents are found on the inside of the meristem."

If seems strange indeed, that while, in the case of other pteridophytes, it is taken as a matter of course that tracheids may retain part of their protoplasmic contents and appear in various stages of development, in *Isoetes* the same condition has been looked upon as an insuperable objection to the tracheid character of the cells in question. It is obviously much more natural to interpret this tissue as a case of arrested development, than to regard it as anything so extraordinary as a combination of xylem and phloem. The fact that we find cases of immature and imperfectly developed secondary xylem in other pteridophytes is more enlightening as a basis of interpretation than is the presence of anomalous secondary thickening in the far-removed *Dracaena*.

The irregular disposition of the thin areas in the pitted tracheids, while not usual in the pteridophytes, is probably more common than has been supposed. GWYNNE-VAUGHAN (11), in his recent work on the tracheae of ferns, calls attention to the irregularities in the Osmundaceae, Botryopteris, and others, illustrating cases of distinctly pitted walls. FARMER and FREEMAN (10) describe a range of structure in the xylem of *Helminthostachys zeylanica* from tracheids "with characteristic bordered pits of an oval or even circular form" to those "in which the pits assimilate to the more scalariform type met with in the majority of ferns." In *Lycopodium* in the primary xylem the thin areas are mostly long and narrow, of the scalariform type, but in the slender tracheids they may be round or oval, giving the tracheid a mottled or pitted appearance. A study of the apices of stems of *Lycopodium* affords an opportunity for a comparison of phloem and young tracheids, a comparison which is of interest as throwing light on

the nature of the pitted cells of Isoetes. In the apices of Lycopodium, where the xylem is not yet mature, it is not difficult to find tracheids that have round or oval pits, and which before they are lignified present a similar appearance to that of the pitted cells of Isoetes. In fact, in *L. pithyoides*, the fully developed sieve cells and the pitted tracheids in which lignification has not yet taken place differ only in the greater regularity of the pitting of the tracheids. If the development of the more slender tracheids of Lycopodium were arrested before lignification had begun, it would be difficult to distinguish sieve cells from tracheids except by position, since both have so nearly the same general appearance and the same reactions to stains. As the disposition of the thickening of the primary tracheids of Isoetes is much less regular than in other pteridophytes, it might reasonably be expected that the secondary xylem would also show irregularities.

One of the reasons given by Russow and subsequent observers for regarding the "prismatic layer" as part phloem, is that it is in direct continuity with the phloem of the leaf traces. I am not disposed to question the nature of the phloem either in the leaves or roots. There is nothing in its position and structure to cause any hesitation about accepting it as phloem. The sieve tubes possess well-defined sieve plates, and the tissue as a whole is so definitely marked off, both in position and development, from the xylem and all adjacent tissues, that there is no apparent reason for questioning its identity as phloem.

There can be no question of the continuity of the old leaf traces and the "prismatic layer" in such cases as are shown in *figs. 19, 20*, but in the case of young leaf traces the point is not so certain. The indications are that the continuity is a result of the overgrowth of the leaf traces by the secondary tissues. *Fig. 17* is a diagram which illustrates a thing that occurs in some if not in all cases where such a continuity exists. In any old stem there are present a very large number of leaf traces, of which only a comparatively few at the top are alive and active. The upper part of the stem is a meristematic region, composed of the meristematic leaf traces and the zone whose lower projection is the cambium. This region is indicated in the diagram by fine dots. The xylem strands of the various leaf traces of that level move in together, forming a more compact region, the vascular axis. The phloem ends in the parenchyma, three or four

cells from the vascular axis, in the region in which the cambium has not yet become defined. As the meristematic region becomes localized, forming the cambium, the parenchyma with which the leaf trace phloem is connected is pushed out farther and farther from the vascular axis by the secondary tissues. The phloem can retain its continuity with this region in three ways: the xylem at the base of the leaf may elongate sufficiently to compensate for the secondary growth in that region; there may be a splitting-apart of the tissues of the bundle, permitting the phloem to slide along the xylem; or the phloem of the leaf trace will be torn apart, leaving one end connected with the "prismatic layer" while the other is carried out into the cortex. Undoubtedly, while the leaf trace is young, there is an adjustment by the first method. It should be noted, however, that the leaf traces which are connected with living leaves are comparatively few in number and are found in that part of the stem in which secondary growth is scarcely observable. At some time in the development of each leaf trace there comes a time when it is no longer capable of extension and is unable to keep pace with the development of the stem. At this point the tissues of the leaf trace give way, and the outer part is carried out into the cortex and finally sloughed off, while the base becomes more or less crushed and remains as a dead stump, which in time may be completely buried in the secondary wood.

While the conditions which have led to the imperfect development of the vascular tissues cannot be known with certainty, among them are doubtless the aquatic habit and the reduction or shortening of the stem. The anatomy of *Isoetes* does not seem to indicate, as SMITH says (p. 324), "that the genus *Isoetes* represents a more primitive type of sporophyte than any other vascular plant," but it supports the view of SCOTT that "the group has clearly undergone reduction from some more complex type, and probably from some highly organized form of lycopod, as indicated by the secondary growth, the marked heterospory, and the somewhat complex organization of the leaves and the root-bearing portion of the axis." As a consequence of a shortening or any reduction in stem development, there would naturally be a reduction in the xylem. This might be either in the amount of the xylem, even to the extent of the entire disappearance of the secondary wood, or there might be a reduction in the development

of the xylem. In the other modern pteridophytes it is apparently the former which has taken place, secondary xylem appearing rarely and in small amounts; and, as HILL (15) infers from a comparison of the examples of pteridophytes showing the phenomenon, it is more probably an example of reduction than a new development. In *Isoetes*, however, the reduction in the bundle has not been limited to the xylem portion, but has extended to the whole of the phloem, both primary and secondary.

The position of *Isoetes*

The phylogenetic connections of *Isoetes* have been discussed in all recent papers, with the great weight of evidence in favor of a lycopod ancestry. The evidence as to its relationship afforded by its anatomy has been taken up recently by BOWER (2), on the basis of the interpretation given by SCOTT and HILL, with the conclusion that *Isoetes* is in its anatomy a lycopod, with a stem structure which can be explained by regarding it as a stunted lycopod. In his eagerness to show a unity of structure in the Lycopodiales, BOWER makes the following statement (p. 339): "Throughout the Lycopodiales the foliar traces are inserted peripherally, and with only a slight local disturbance upon the periphery of the cauline xylem core." In view of the questionable existence of a cauline portion in the xylem core, there seems to be little justification for so sweeping a statement. It is in this very thing that *Isoetes* differs markedly from other adult lycopods, although the difference is not of such a character as to make the relationship doubtful. The difference is correlated with the stunted habit, and such differences of body habit have never been admitted to have great weight in determining the larger groupings. The stunted habit of stem is not limited in Lycopodiales to *Isoetes*, but it occurs also, though of very different type, in *Phylloglossum*.

Even among those who recognize the strong lycopod affinities of *Isoetes*, it is suggested occasionally that it might be advisable to separate *Isoetes* from the lycopods and establish a new order, *Isoetales*. The present tendency seems to be toward a multiplication of orders, so that it may be well to consider the desirability of it in this case.

The closest connection of *Isoetes* is, as has been frequently pointed out, with the *Lepidodendreae*, although it has many points in com-

mon with modern lycopods. The spore-producing members, in structure and development, are unquestionably of the lycopod type, and as such present no obstacle to the retention of the group in the Lycopodiales. With reference to the anatomy my work would seem to strengthen the position of Isoetes in the Lycopodiales. The irregularities of its structure are not of such a nature as to isolate the group. Aside from the possible lack of a cauline portion in the stele, the irregularities of its anatomy are limited to the absence of primary phloem in the stem; the absence of secondary phloem; the lack of differentiation into protoxylem and metaxylem in the stem; the large amount of parenchyma in the secondary wood; and the imperfect development of the wood.

The absence of primary phloem seems to be characteristic of Isoetes. There are no indications of it in the four species described in this paper, and SCOTT and HILL say of *I. Hystrix* "that it is not possible to identify primary phloem with certainty." Although the absence of primary phloem is recorded for juvenile pteridophytes (e.g. *Matonia pectinata*) by TANSLEY and LULHAM, (23, p. 482), so far as is known it is present in the stem of all other adult pteridophytes. In this point, then, Isoetes stands alone. The presence of phloem in the leaf traces and roots, and the collateral arrangement of the bundle in the lower part of the leaf trace indicates a descent from a line in which the phloem is present in the stem as a layer around the xylem. It is difficult to tell what importance to attach to the tendency of the leaf trace to become concentric in the middle and upper part; and to the occasional occurrence of mesarch xylem in the leaf trace.

In regard to the next point, the absence of secondary phloem in a stem with a cambium, the isolation of Isoetes is less certain. SCOTT (20, p. 167) in discussing the secondary growth of the Lepidodendreae says: "Although the presence of primary phloem can be recognized with certainty, some doubts have been expressed as to the production of secondary phloem by the cambium." It may be well to remember, too, that there are cases among the modern pteridophytes in which secondary xylem is produced, but no secondary phloem has been observed. This suggests that when reduction occurs in a form with secondary thickening, the disappearance of the phloem precedes that of the xylem.

The next peculiarity of the stem anatomy, the absence of differentiation into protoxylem and metaxylem in the vascular axis, is so obviously related to the stunted habit of the stem that it contributes nothing to a discussion of the position of *Isoetes*.

In the peculiarities of the structure of its secondary wood, *Isoetes* finds its nearest prototype in certain *Lepidodendreae*, as has been pointed out by previous writers. The forms which present the greatest similarity of structure are *Lepidophloios fuliginosus* and *Lepidodendron obovatum*. According to SCOTT (21) the cambium in *Lepidodendron obovatum* produces parenchyma only and no tracheids; but the cambium in *Lepidophloios fuliginosus* produces either secondary parenchyma only, or secondary parenchyma in which are imbedded groups of tracheids. Certain species of *Isoetes* present a close approximation to both conditions. In some species the secondary xylem is almost wholly parenchymatous, with no well-formed tracheids and only a few immature tracheids. In other cases there are groups of tracheids associated with the parenchyma, and in addition a certain amount of immature tracheid tissue. *Isoetes* accordingly differs from these two members of the *Lepidodendreae* only in the presence of immature tracheids in the secondary xylem. It is not improbable that future work in the *Lepidodendreae* may bring to light a similar situation in that group.

In point of anatomy, then, there seems to be no adequate ground for the separation of *Isoetes* from the *Lycopodiales*.

The strongest argument for the establishment of a separate order has been drawn from the gametophyte generation, in the presence of a multiciliate sperm. While this is a character of great importance, we should consider the extent of our evidence before attaching too much weight to it. It must not be forgotten, moreover, that lycopod sperms occasionally depart from the biciliate type. BRUCHMANN (4, p. 32) speaks of the occasional occurrence of sperms with three cilia in *Lycopodium clavatum*. If we reflect that our knowledge of the sperms of the modern genus *Lycopodium* is limited to those of a few species, and that we have no knowledge at all of the sperms of the more closely related *Lepidodendreae*, we may be less inclined to regard the character of the sperms as preponderant in determining the position of *Isoetes*.

Summary

1. The vascular axis is a non-medullated monostele, composed of tracheids and parenchyma. There is no differentiation into protoxylem and metaxylem.

2. There is no primary phloem in the stem. It is found in the leaf traces and root bundles only.

3. The cambium gives rise to cortex on the outside and secondary xylem on the inside. The so-called "prismatic layer" is secondary xylem. The cambium does not form phloem.

4. The secondary xylem consists of various combinations of the following types of cells: (a) Spiral and annular tracheids. (b) Immature tracheids, slightly lignified, with irregular rings or spiral thickenings. (c) Immature tracheids, unlignified, nucleate or enucleate, with irregular rings or spiral thickenings. (d) Immature tracheids, nucleate or enucleate, with slightly thickened, pitted walls. (e) Parenchyma cells, which may have little protoplasm and small nuclei, or abundant protoplasm and large nuclei.

5. The secondary xylem of *I. Nuttallii* shows zonation. *I. echinospora* var. *Flettii* and *I. Tuckermanni* var. *Harveyi* do not. *I. melanopoda* shows it occasionally in old stems. Starch does not occur in the secondary xylem except in the parenchyma zones of *I. Nuttallii*.

6. The root bundles are collateral and monarch. The protoxylem is found on the side away from the phloem and toward the center of the stem, i. e., it is endarch.

7. The leaf traces are collateral, but tend to become concentric in the middle and upper part of the leaf. The xylem portion undergoes great reduction above the sporangium, but the phloem is not reduced correspondingly. The sieve plates are transverse.

8. Near the vascular axis the leaf trace does not show differentiation into protoxylem and metaxylem. In the outer part of the cortex and in the region of the sporangium it is usually exarch. In *I. Nuttallii* it is occasionally mesarch above the sporangium and in the region of the sporangium.

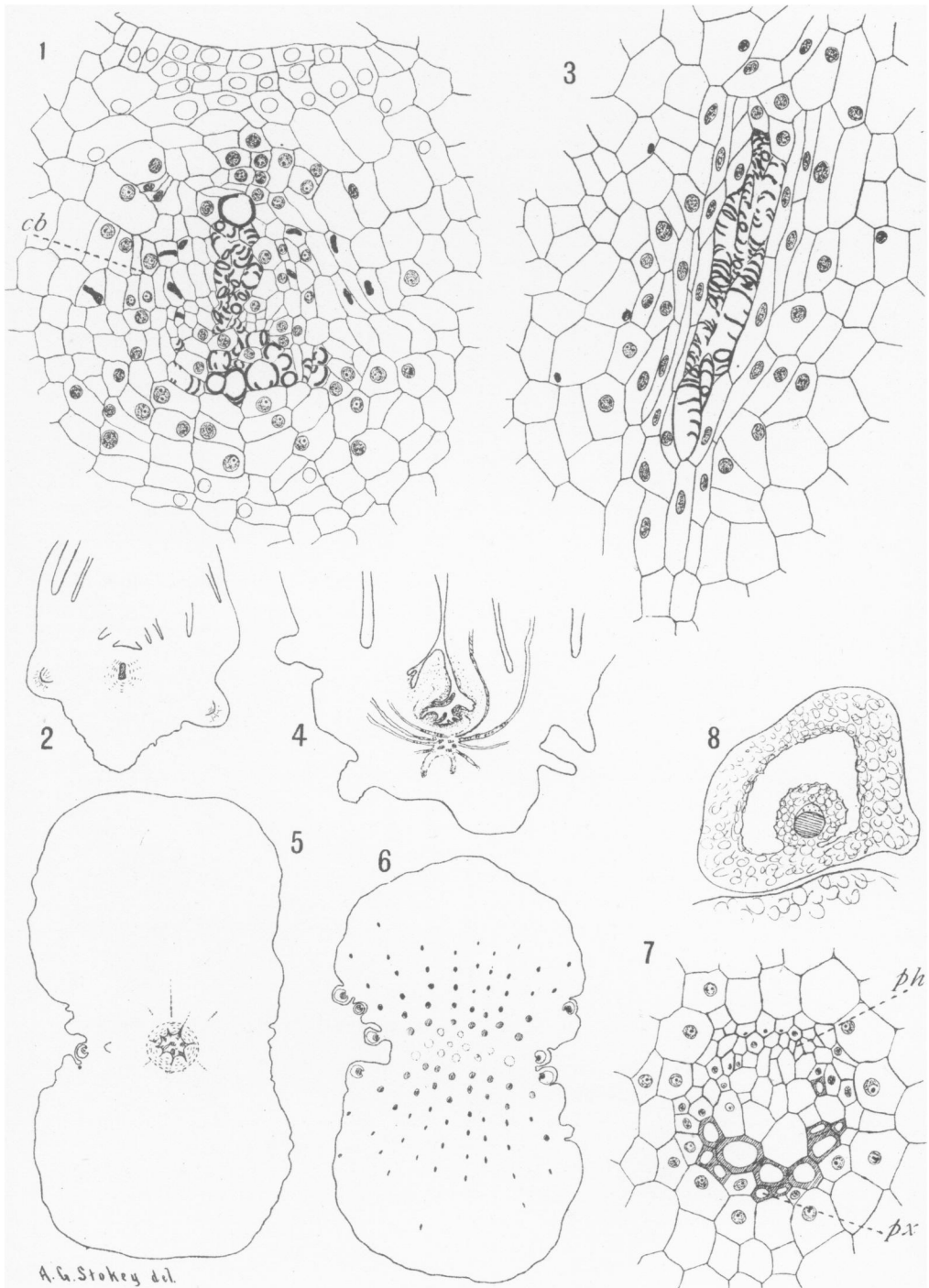
This investigation was conducted at the University of Chicago under the direction of Professor JOHN M. COULTER and Dr. W. J.

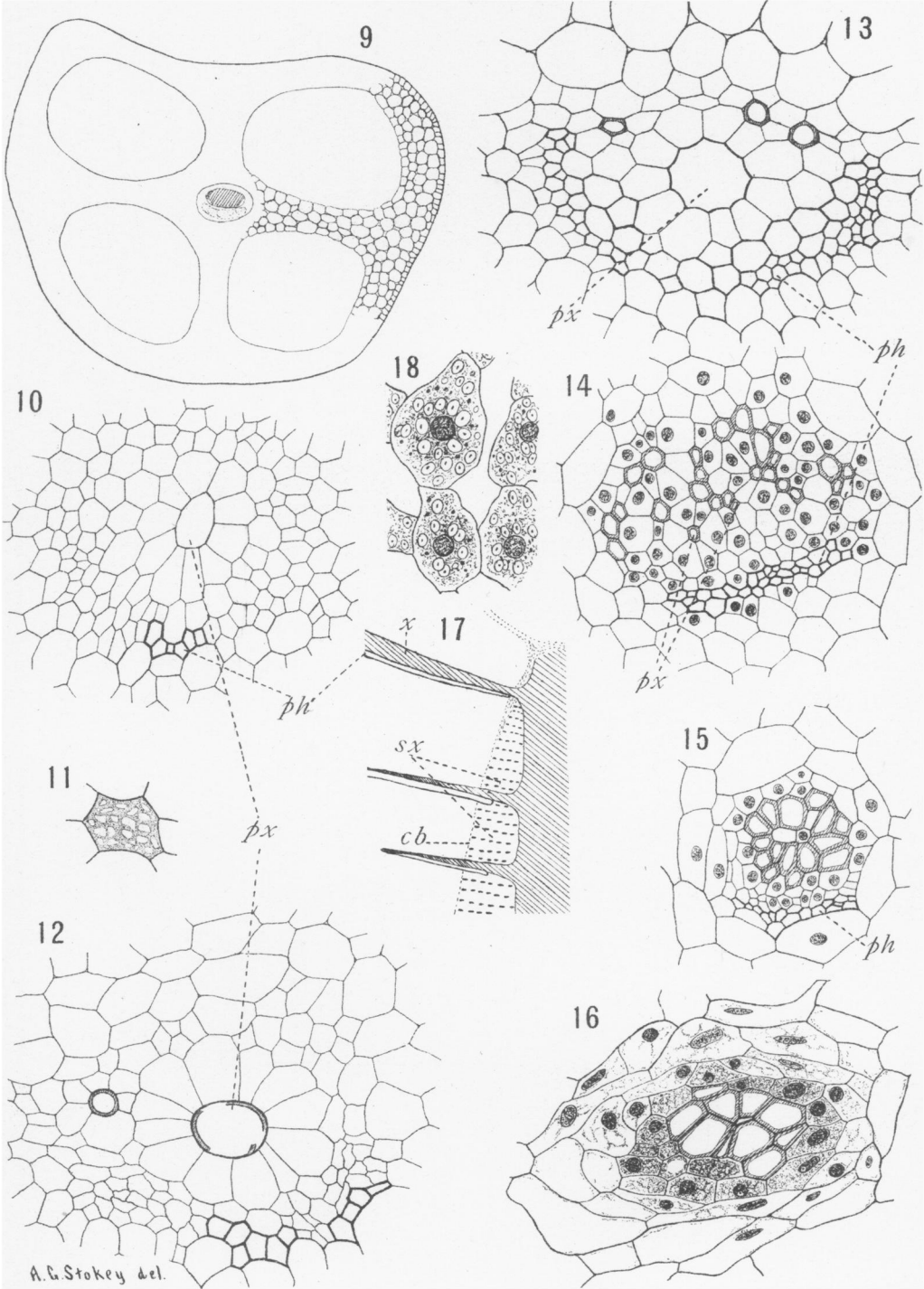
G. LAND, of whose advice and encouragement I wish to express my keen appreciation.

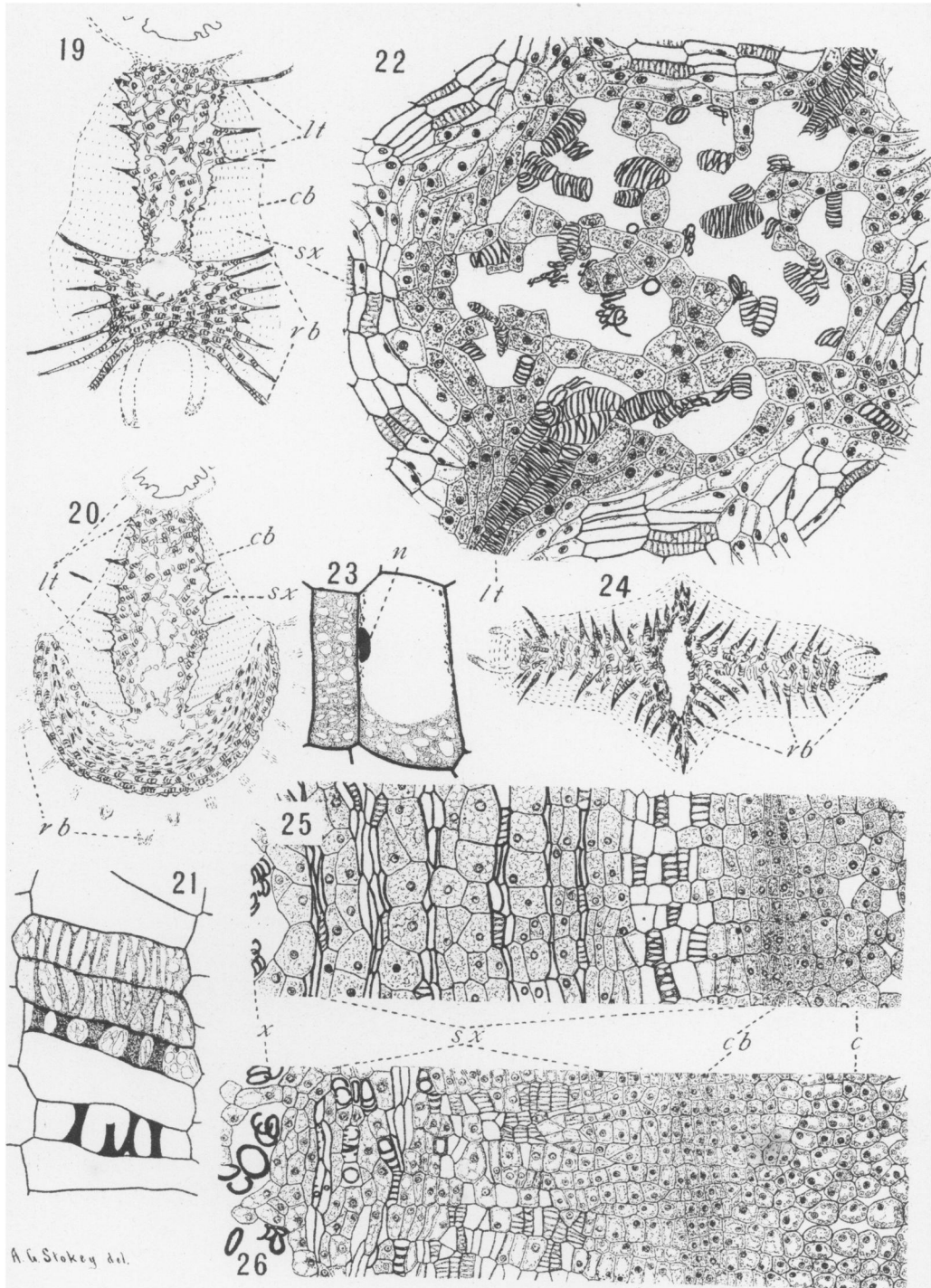
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EXPLANATION OF PLATES XIX-XXI

PLATE XIX

Figs. 1-4.—*I. echinospora* var. *Flettii*. Figs. 5-8.—*I. melanopoda*

FIG. 1.—Longitudinal section of the stele of young plant cut in the plane of the furrow. $\times 380$.

FIG. 2.—Longitudinal section of the stem of young plant cut in the plane of the furrow. $\times 37$.

FIG. 3.—Cross-section of the stele of a young plant. $\times 380$.

FIG. 4.—Longitudinal section of a young plant cut across the furrow. $\times 22$.

FIG. 5.—Cross-section of the stem of old plant in the leaf-trace region. $\times 6$.

FIG. 6.—Cross-section of stem below the stele showing root bundles in the cortex. $\times 6$.

FIG. 7.—Cross-section of root bundle before it has left the stem. $\times 380$.

FIG. 8.—Cross-section of mature root. $\times 48$.

PLATE XX

Figs. 9, 10, 12-16.—*I. echinospora* var. *Flettii*. Fig. 11.—*I. Nuttallii*

FIG. 9.—Cross-section of mature leaf near the middle. $\times 37$.

FIG. 10.—Cross-section of bundle of young leaf near the tip. $\times 380$.

FIG. 11.—Sieve plate from sieve tube. $\times 810$.

FIG. 12.—Cross-section of bundle of a young leaf near the middle. $\times 380$.

FIG. 13.—Cross-section of bundle of mature leaf cut just above ligule. $\times 380$.

FIG. 14.—Cross-section of leaf trace in cortex just below base of leaf. $\times 260$.

FIG. 15.—Cross-section of leaf trace in cortex half-way between leaf and vascular axis. $\times 380$.

FIG. 16.—Cross-section of leaf trace near the vascular axis. $\times 380$.

FIG. 17.—Diagram to illustrate the relation of leaf traces to secondary wood.

FIG. 18.—Cells of cortex with starch grains. $\times 380$.

PLATE XXI

Figs. 19, 20, 22.—*I. echinospora* var. *Flettii*. Figs. 21, 24, 26.—*I. melanopoda*

Fig. 25.—*I. Nuttallii*. Fig. 23.—*I. Tuckermanni* var. *Harveyi*

FIG. 19.—Longitudinal section of vascular bundle of old plant cut across the furrow. $\times 22$.

FIG. 20.—Longitudinal section of vascular bundle of old plant cut in the plane of the furrow. $\times 22$.

FIG. 21.—Tracheids from secondary xylem at different stages of development. $\times 810$.

FIG. 22.—Cross-section of vascular axis of old plant. $\times 175$.

FIG. 23.—Tracheids from secondary xylem; the cell on the right is sectioned obliquely. $\times 810$.

FIG. 24.—Cross-section of vascular axis through the root region; the long axis is in the plane of the furrow. $\times 22$.

FIG. 25.—Cross-section of stem from the edge of the primary xylem to the cortex, showing zonation in secondary xylem. $\times 450$.

FIG. 26.—Cross-section of the stem from the edge of the primary xylem to the cortex. $\times 350$.